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Development and Validation of a Method for the Determination of (*E*)-Resveratrol and Related Phenolic Compounds in Beverages Using Molecularly Imprinted Solid Phase Extraction

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ABSTRACT: A molecularly imprinted polymer was prepared using (*E*)-resveratrol as template and was evaluated for multicomponent multiclass analysis of polyphenolic compounds in complex matrices such as natural and alcoholic beverages. Chromatographic evaluation of the polymer exhibited high selectivity for (*E*)-resveratrol and its structural analogues, quercetin, and other flavonoids. An analytical procedure based on molecularly imprinted solid phase extraction (MISPE) and high-performance liquid chromatography coupled to UV detector was developed and validated for determination of (*E*)-resveratrol and quercetin in wine and fruit juice samples. The specific binding capacity of the MIP was estimated as 80 $\mu g\ g^{-1}$ polymer by the cartridge test. MISPE sample pretreatment allows an excellent sample cleanup, enormously decreasing the number of coextracted potentially interfering compounds. Under the described conditions, by extracting 2 mL samples a clean extract is obtained and (*E*)-resveratrol and quercetin could be easily identified at concentration levels of, respectively, 1.5 and 7.0 $\mu g\ L^{-1}$.

KEYWORDS: *molecularly imprinted polymer, solid phase extraction, flavonoids, (E)-resveratrol, quercetin, wine, red fruits juice, lingonberry*

INTRODUCTION

The relevant amount of antioxidant compounds in foods and natural and alcoholic beverages such as wine and fruit juice is often referred to as having a role in the so-called "French paradox" (the lower-than-expected coronary mortality in France) as well as other positive effects on health.[1,2] Among several other compounds, (*E*)-resveratrol and quercetin are often investigated and referred to as responsible for these effects.[3]

(*E*)-Resveratrol (*trans*-3,5,4′-trihydroxystilbene) has been identified as one of the major active compounds of the stilbene phytoalexins,[4] isolated at first from the roots of white hellebore and later from *Polygonum cuspidatum*, a member of the family Polygonaceae. (*E*)-Resveratrol has been found in grapes, wine, peanuts, and peanut products so far,[5] and it has been associated with several beneficial health effects such as antioxidative, anti-inflammatory, and estrogenic effects as well as anticancer and chemopreventive activities.[5,6] The effects of (*E*)-resveratrol observed in vivo and related possible evolutionary explanations, as well as development of human therapeutics based on either (*E*)-resveratrol itself or new, more potent compounds that mimic its effects, have been recently reviewed.[7] Despite its particularly low bioavailability and rapid clearance from the circulation,[8] there is growing evidence that (*E*)-resveratrol can prevent or delay the onset of cancer, heart disease, ischemic and chemically induced injuries, diabetes, pathological inflammation, and viral infection.[7] On the other hand, because of the toxic effects observed when (*E*)-resveratrol was administered at or above 1 g (kg body weight)$^{-1}$, higher doses to improve efficacy might not be possible.[9] Moreover, due to the high current cost of (*E*)-resveratrol for a human weighing 75 kg, a daily dose of 100 mg (kg body weight)$^{-1}$ would cost U.S. \$6800 year^{-1}.

Therefore, there is a great interest in developing analogues with higher bioavailability or finding new, more powerful compounds that mimic its effects.

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one) is a flavonol widely occurring in plants and significantly present in red wine and other fruit products. As well as for (*E*)-resveratrol several biological actions of quercetin including protection of LDL cholesterol against oxidation,[10] promotion of endothelial vasorelaxation,[11] and reduced risk of cancer[12] have been reported. In particular, a synergistic effect between ethanol and the most abundant wine polyphenols, quercetin and resveratrol, in inhibiting the inducible nitric oxide synthase pathway involved in the damage of vascular walls and DNA has been demonstrated.[13] Finally, in a recent study, (*E*)-resveratrol and quercetin have been suggested as indirect activators of the aryl hydrocarbon receptor (AHR).[14]

Prompted by the increasing interest in natural polyphenolic antioxidants, a large number of analytical procedures were recently suggested.[15−17]

Molecularly imprinted polymers (MIPs) are functional polymers generated by molecular imprinting, an efficient method for producing functional materials equipped with selective identification characteristics. The technique consists of self-assembly of a functional monomer and a template molecule in solution followed by copolymerization of the functional monomer with an excess of an appropriate cross-linking monomer. After removal of the template, the resulting polymer exhibits high affinity for the molecule used as template and structural analogues. In the past decade MIPs used for solid phase extraction (MISPE) have been successfully applied to solve

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several challenging issues in food, biological, and environmental analyses[18] and became commercially available. In recent literature syntheses of flavonol[20,21] and quercetin imprinted polymers[22−26] are also reported.

Very recently MIP of (*E*)-resveratrol has been designed with molecular modeling to determine the optimal amount of template to associate with the functional monomer for the formation of the polymer[4] and evaluated for the isolation and concentration of resveratrol, caffeic acid, (−)-catechin, and (*E*)-piceid in peanut byproducts.[19] In previous papers this MIP was evaluated for the extraction of (*E*)-resveratrol from *P. cuspidatum*.[27−29]

The purpose of this study was the evaluation of a (*E*)-resveratrol imprinted polymer for the extraction of natural antioxidants from plants, food matrices, and foodstuffs not previously investigated such as alcohols. The feasibility of such an approach was evaluated by comparing the behavior of a few model compounds belonging to different chemical classes which in some cases have structural or functional analogies to resveratrol. The potentiality of using such polymers for multiclass (multiresidue) analysis of selected polyphenolic structures from complex matrices was finally evaluated by analyzing (*E*)-resveratrol and quercetin in beverages such as wine and fruit juice.

■ MATERIALS AND METHODS

Safety. Performing synthesis inside a glovebox presents several advantages especially for reducing oxygen presence and the risks for operators. In this study MIP synthesis was realized in a homemade glovebox using a 120 × 100 × 60 cm PVC vacuum bag for cloths equipped with a sealing closure and an evacuation valve to easily deflate the bag by removing air oxygen. The glovebox was set under a cabin fume with all of the required reagent and devices necessary inside. The bag was then sealed, and air was evacuated using a water vacuum pump. The PVC bag was then inflated with nitrogen. An interesting feature of this setup is the possibility of removing air without relevant pressure reduction.

Chemicals and Materials. Acetonitrile HPLC-MS grade, ethanol ACS-For Analysis Reag. Ph. Eur. Reag. USP, methanol HPLC-MS, acetone RS for HPLC, acetic acid, formic acid, ethylene glycol dimethacrylate (EGDMA), and 4-vinylpyridine (4-VP) were obtained from Carlo Erba (Milan, Italy). HPLC grade purified water was obtained by using a Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA). (*E*)-Resveratrol (98%) was purchased from Farmalabor (Canosa di Puglia (BT) Italy). Acetonitrile and methanol employed for the HPLC-UV-MS analyses were of HPLC super gradient quality (Romil Ltd., Cambridge, UK). PBS phosphate buffer powder, 0.1 M, free radical iniziator α,α'-azoisobutyronitrile (AIBN), genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one) synthetic 98% HPLC powder, apigenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one) 95% HPLC powder, curcumin ((1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) powder from *Curcuma longa* (turmeric), (*S*)-ketoprofen ((*S*)-2-(3-benzoylphenyl)propionic acid) 99%, and quercetin dihydrate 98% HPLC powder were obtained from Sigma-Aldrich. Syringe filter 0.45 μm PVDF (Alltech Italia Srl, I-20017 Passirana di Rho MI, Italy). Aglianico red wine, blended "red fruits" juices, and lingonberry juice drink were collected at local markets.

Standard Solution and Sample Preparation. A stock standard solution of 1 mg mL^{-1} of each compound was prepared in methanol and stored at 4 °C in darkness. Working standard solutions were prepared by diluting the stock standard solutions with purified water or mobile phases.

Prior to extraction, real samples were filtered using PVDF 0.45 μm syringe filters. One milliliter of filtered samples was then diluted with 3 mL of HPLC water and 1 mL of pH 7 phosphate buffer (PBS), 0.1 mol L^{-1}.

Synthesis of (*E*)-Resveratrol MIP. The MIP was prepared by bulk polymerization. The template (*E*)-resveratrol (1 mmol, 228 mg) was dissolved in acetonitrile/ethanol (5 mL, 5:1 v/v) in a glass tube with a screw cap. The functional monomer 4-vinylpyridine (0.322 mL, 3.0 mmol) was added, and the mixture was sparged with N_2 gas and sonicated for 10 min. Then the cross-linker EGDMA (2.314 mL, 15 mmol) was added followed by the free radical iniziator AIBN (51 mg, 0.31 mmol) previously dissolved in acetonitrile:/ethanol (1 mL, 5:1 v/v). This prepolymerization mixture was sparged with N_2 gas for 5 min and then placed in a thermoblock at 50 °C for 24 h and a further 24 h at 60 °C. The same procedure without added template was used to prepare a nonimprinted polymer for control experiments (NIP). The glass tubes were shaken and rotated periodically to ensure homogeneous polymerization. The polymers were then removed from the glass tubes, crushed in a mortar, and ground using a ball mill for 30 min at speed 5. The ground particles were subsequently sieved using a sieve shaker (Retsch AS 200 control 'g') (amplitude, 1.50 mm g^{-1}; interval time, 30 s; time, 10 min). In all experiments particles of 75−106 μm size were employed. The polymer was then sedimented to eliminate fines. The sedimentation was done in acetone by repeated cycles of suspension, decanting the supernatant and adding fresh acetone to the precipitated particles. The (*E*)-resveratrol template was removed from the MIP by intense washings in methanol containing 10% acetic acid with gentle stirring. The washings were monitored by HPLC-UV at 306 nm and continued until the template could no longer be detected. Polymer particles were then washed with methanol to remove traces of acetic acid and dried in a vacuum for 48 h.

Chromatographic Evaluation of MIP Retention and Imprinting Factors (IF). The molecular recognition properties of produced polymers were evaluated by using the polymers as HPLC stationary phases. MIP and NIP particles (75−106 μm) were packed into a stainless steel (20 mm × 2 mm i.d.) Direct-Connect Refillable Guard Column by slurry packing method using methanol up to a constant pressure of 20 mPa. The amount of dry polymer packed was measured as 19.0 ± 0.8 mg.

To remove any trace of template bleeding, the packed columns were washed with methanol at 100 μL min^{-1} until a stable baseline (306 nm) was obtained. After equilibration, 50 ng of selected model compounds standard solution was injected at the same flow rate. Each analysis was repeated three times to evaluate repeatability. The experiments were repeated using different solvents and combination of solvents as mobile phase. Methanol was used to wash the column after each injection. Detection was performed at a suitable wavelength for each analyte. Column void volumes were measured by injecting 5 μL of acetone 1% v/v. All of the measurements were performed at 25 °C.

The retention factors were calculated from the equation $k' = (t_r - t_0)/t_0$, where t_r and t_0 are the retention times of the analytes and acetone, respectively. The IF was then calculated as the ratio of the MIP/NIP retention factors.

MISPE Cartridge Preparation. The polymer particles, 75−106 μm in diameter, were suspended in methanol. MIP and NIP cartridges were prepared by packing 50 mg of the respective polymer particles into empty 3 mL SPE cartridges (Supelco, Bellefonte, PA, USA) and secured by polyethylene frits at the top and bottom. The extraction was performed using a 12 port vacuum manifold from Phenomenex. The eluent was forced to pass through the system by regulating the vacuum to approximately 15 kPa to obtain a flow rate of 1 mL min^{-1}.

To evaluate the repeatability of the extraction protocol, each experiment was repeated in triplicate using three different cartridges. Before each experiment, the cartridges were conditioned with 2 mL of methanol and 2 mL of acetonitrile. After sample extraction, the cartridges were washed with 3 mL of acetonitrile to remove interfering compounds. Analyte elution was performed by passing 6 mL of a solution containing 10% acetic acid in methanol.

After the extract had been exhaustively dried at room temperature under a gentle stream of nitrogen, the residue was reconstituted with 500 μL of water/methanol (1:1 v/v) and 5 μg of genistein was added as internal standard. No losses were observed during the evaporation step. Samples were analyzed by HPLC-ESI-MS when confirmation was

necessary. Regeneration of the SPE columns was performed by washing the polymer with 2 mL of methanol followed by 2 mL of acetonitrile.

HPLC Analysis. The quantification of phenolic compounds was performed using an HPLC system consisting of an Accela pump (Thermo Finnigan, San Jose, CA, USA) and an Accela autosampler coupled to a UV (Varian) variable wavelength UV−vis detector. LC-ESI-MS confirmatory analyses were performed using an Agilent (Palo Alto, CA, USA) 1100 HPLC system coupled to an Applied Biosystems API2000 triple-quadrupole instrument (Applied Biosystems, Foster City, CA, USA).

Separations were carried out with a Luna 2.5 u C18 (2)-HST reverse phase column (100 × 2.0 mm) equipped with a Security Guard HPLC Guard Cartridge System (Phenomenex) using water (solvent A) and acetonitrile (solvent B) both containing 0.1% (v/v) formic acid as mobile phase at a flow rate of 200 μL min^{-1}. The injection volume was 5 μL. The initial percentage of solvent B was 20%, and this was linearly increased to 90% over 15 min. (*E*)-Resveratrol and quercetin were detected respectively at wavelengths of 306 and 270 nm, and data acquisition was performed using Clarity DataApex software.

Quantitative analysis was performed using the peak area ratio of analytes to internal standard. Percentage recoveries were determined by comparing the calculated amount versus the amount used in the spike.

Standard solutions for the calibration curve were prepared by dilution of the stock solution with a concentration of 1 g L^{-1} in methanol in a solution of methanol/water 1:1 (v/v).

RESULTS AND DISCUSSION

Chromatographic Evaluation of MIP Selectivity and Rebinding Ability. Sample preparation is the most critical step approaching the analysis of very complex matrices such as natural products. Even using MS detection the presence of millions of possible interfering compounds makes really difficult the identification of target compounds and very unreliable the quantitative determination.[30] For this reason sample cleanup is often required through several additional steps. Selectivity of a stationary phase is determined as the ratio of the target analyte retention factor and the possible interfering compounds retention factors. As long as the retention factors are different, it will be easier to get rid of unsettling compounds. The availability of an efficient selective material for solid phase extraction might greatly simplify the procedure, reducing costs and improving quality parameter of the analytical procedure. Selectivity of (*E*)-resveratrol MIP was investigated by determining retention factors and imprinting factors of selected model compounds (Figure 1) on both MIP and NIP packed in a short HPLC column. Different solvents were used to verify MIP efficacy of such solvents as washing or elution phase for the considered analytes. Results are summarized in Table 1.

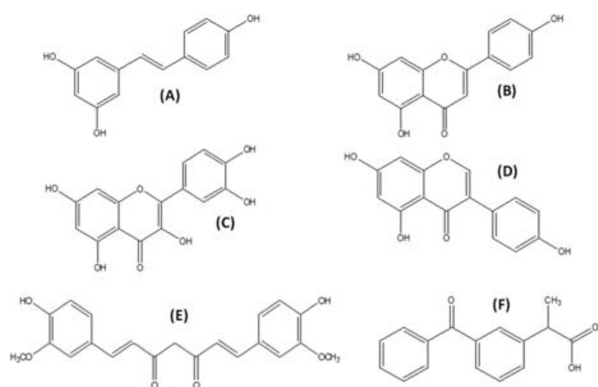

Figure 1. Molecular structures of selected model compounds: (A) (*E*)-resveratrol (*trans*-3,5,4′-trihydroxystilbene); (B) apigenin; (C) quercetin; (D) genistein; (E) curcumin; (F) ketoprofen.

Table 1. Chemical Properties and Characteristics of Selective Retention on MIP for Model Compounds[a]

compd	log P^b	pK_a^{b}	IF ACN	IF MeOH
resveratrol	3.08	9.07	nc[c]	8.30 ± 0.1
quercetin	1.48	7.17	nc	2.64 ± 0.2
apigenin	3.02	7.12	nc	1.67 ± 0.04
genistein	2.84	7.63	0.813 ± 0.01	0.41 ± 0.01
ketoprofen	3.01	4.25	0.735 ± 0.08	0.32 ± 0.1
curcumin	3.20	8.11	1.23 ± 0.01	0.56 ± 0.01

[a]Octanol−water partition coefficient, acidity in water, and imprinting vactor (calculated as MIP/NIP retention factors ratio) in acetonitrile and methanol (elution solvent). [b]Calculated using Advanced Chemistry Development (ACD/Laboratories) software V11.02 (1994−2012 ACD/Laboratories). [c]Not calculable.

Selection of model compounds was done bearing in mind a pharmacophore model containing four common chemical features in antioxidant agents: one aromatic ring and three hydrogen bond acceptors.[31,32] The template itself and two more compounds (quercetin and apigenin) had these specific features. Three more molecules were also evaluated for MIP retention effects as control compounds to emphasize the effects of nonspecific interaction such as ion exchange or nonspecifically oriented hydrogen bond: genistein is an isoflavonoid then based on 3-phenylchromen-4-one structure instead of 1-phenylchromen-4-one; the phenols of curcumin, a well-known natural phenolic antioxidant, are connected by two α,β-unsaturated carbonyl groups and the diketones form stable enols and enolates; ketoprofen is a compound with molecular weight and raw formula similar to those of (*E*)-resveratrol but different molecular structure and physical−chemical properties: it is more acidic and, as well as resveratrol, it contains both H-bond donor and acceptor sites.

It has been often shown that H-bond-based MIPs exhibit higher selectivity in nonprotic solvents such as acetonitrile.[33] Chromatographic rebinding experiments results (see Table 1) demonstrate that molecules with very similar pharmacophore triangles were not eluted in acetonitrile over 120 min, making, from a practical point of view, the calculation of the retention factor impossible. Moreover, even in a protic solvent such as methanol, the template molecule is hardly displaced from selective interaction sites (IF 8.30) and molecules with very similar H-bond donor stereochemistry are still significantly retained (see Figure 2).

On the other hand, it has been often observed that analytes can be retained by MIPs and NIPs through nonspecific interactions assisted mainly by solvophobic effects.[34] Differences in polymer characteristic (i.e., surface area) might be responsible for slightly stronger nonspecific retention of NIPs generating IF < 1 for molecules that are very different from the template.

Evaluation of Selectivity and Rebinding Capacity of MISPE Cartridges. To investigate the feasibility of using (*E*)-resveratrol MIP to develop a multicomponent multiclass method for polyphenolic compounds in food matrices and beverages, the two most abundant polyphenolic compounds, namely, (*E*)-resveratrol and quercetin, were elected for evaluating the polymer selective retention effects.

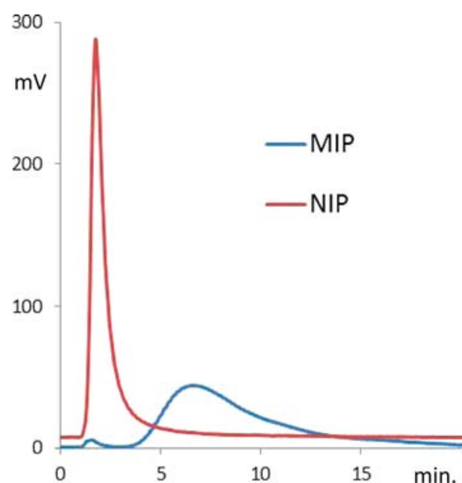

Figure 2. Chromatographic determination of imprinting factors on short HPLC column filled with MIP (blue line) and NIP (red line) polymers. Unless eluted in a relatively short time, (*E*)-resveratrol is selectively retained even in the elution solvent (methanol).

To estimate the rebinding ability of the polymer, 50 mg cartridges were packed using both MIP and NIP particles in the size range 75−106 μm and used for breakthrough experiments.

Breakthrough volume was investigated by extracting water and acetonitrile samples up to, respectively, 5 and 100 mL sample size. Acetonitrile could be used as solvent for extracting polyphenols from solid matrices or washing filtrate from beverages. As expected from the chromatographic retention experiments, no relevant losses of these two analytes occurred for both solvents (see Table 2).

Table 2. Breakthrough Experiments Performed Using 50 mg MISPE Cartridges As Described under Materials and Methods To Extract Different Solvent Samples Spiked with 2 μg of Resveratrol

	water		acetonitrile	
sample vol (mL)	recovery (%)	SD	recovery (%)	SD
5	101.6	4.8	101.0	3.7
10	nd[a]		82.7	10
25	100.3	2.1	78.0	4.4
50	99.6	3.2	72.8	1.7
100	100.0	6.7		

[a]Not determined.

The nonspecific hydrophobic interactions are considerably reduced in acetonitrile, allowing the elution of substances not specifically retained by the MIP affinity binding sites. A protic solvent such as methanol weakens the hydrogen bond, reducing the retention effect and allowing the elution of several analytes (results not shown). The presence of acetic acid was necessary to achieve quantitative elution of most retained compounds (such as (*E*)-resveratrol and quercetin) from the MISPE column with a small amount of mobile phase.

Validation of MISPE HPLC-UV Method for the Analysis of (*E*)-Resveratrol and Quercetin in Beverages. The potential use of MISPE cartridges for determining polyphenolic compound content in beverages was assessed by performing selected model compound recovery experiments from different solvents and different concentration levels. As expected from preliminary experiments, no breakthrough was observed from water samples up to 5 mL of acetonitrile and 100 mL of water (see Table 2). In fact, in water, nonspecific interactions play a major role in analyte retention mainly depending on water solubility, log P and pK_a values. In acetonitrile, experiments were performed by spiking samples at 2 μg (50% of calculated capacity), and no losses occurred at 5 mL volume, confirming preliminary experiments used to determine the washing step volumes in the MISPE procedure. At higher sample volume some breakthrough occurred with moderate increase by increasing the sample volumes according to typical MIP binding isotherm. Table 3 reports separate recovery experiments for (*E*)-resveratrol and quercetin, confirming the excellent performances of MISPE cartridges far from saturation effects. In such conditions recoveries were >90% and standard deviation <6%. Linearity was investigated in the range of 0.5−5 μg mL^{-1}. A Pearson correlation coefficient of R^2 = 0.9997659 was obtained for the curve of (*E*)-resveratrol (y = 188.5573x), and R^2 = 0.999323 was obtained for the curve of quercetin (y = 50.39619x). Limits of detection, defined as 3 times the signal-to-noise ratio, were estimated at 0.15 ng injected for (*E*)-resveratrol and 0.70 ng for quercetin. In these conditions, considering a sample size of 2 mL and concentrating the eluate up to 100 μL, method detection limits (MDLs) were estimated as 1.5 and 7.0 μg L^{-1}, respectively, for (*E*)-resveratrol and quercetin.

Recovery and Analysis of (*E*)-Resveratrol and Quercetin in Real Samples. To carefully evaluate the efficacy of MISPE cartridges in the extraction from complex matrices, recovery of (*E*)-resveratrol and quercetin was performed from spiked samples of wine and "red fruits" juice. Excellent reproducibility was obtained in both cases as reported in Table 4. The binding capacities of the MISPE cartridges were assessed by recovery measurements at high concentrations. The MISPE cartridges showed excellent stability properties. The same three cartridges were used for all of the experiments described in this work. The performances in the last experiments were confirmed by three freshly prepared cartridges. No relevant differences were observed.

The advantages of using MISPE are clearly shown in Figures 3 and 4, where the chromatograms corresponding to, respectively,

Table 3. Recovery Experiments Using 50 mg MISPE Cartridges at Different Concentration Levels from 5 mL Water and Acetonitrile Samples

	resveratrol				quercetin			
	water		acetonitrile		water		acetonitrile	
spike (μg)	recovery (%)	SD	recovery (%)	SD	recovery (%)	SD	recovery (%)	SD
0.5	98.2	0.68	98.6	2.8	97.5	3.2	91.4	5.8
1	101.6	4.8	99.9	3.4	98.7	1.6	98.8	4.8
2	101.6	4.8	100.9	3.7	86.4	0.15	85.9	4.6
4	100.7	3.7						

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Table 4. Evaluation of Competitive Effects from Complex Matrices[a]

	resveratrol				quercetin			
	wine		red fruits juice		wine		red fruits juice	
spike (μg)	recovery (%)	SD	recovery (%)	SD	recovery (%)	SD	recovery (%)	SD
0.5	95.2	3.6	92.3	2.3	91	3.8	97.4	1.1
1			99.5	1.7	88.2	1.5	98.8	3.1
1.5	97.4	1.2	99.3	1.5	94.7	1.2	94.7	1 0.3
2	96.5	1.5	99.5	2.5	95.4	0.8	95.4	0.62
3	100.3	0.095						

[a](*E*)-Resveratrol and quercetin recoveries from real samples spiked at different concentration levels.

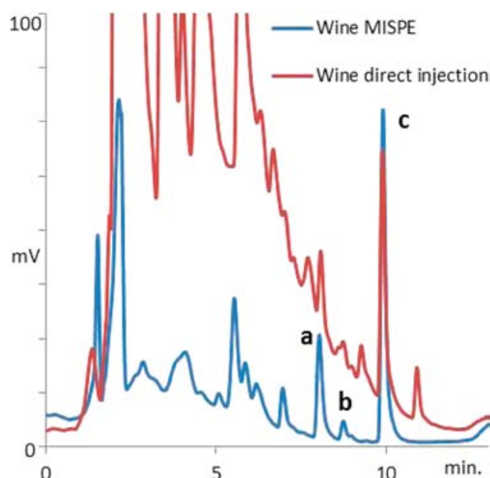

Figure 3. Chromatograms of red wine samples: (red line) untreated red wine; (blue line) time MISPE enriched extract of same red wine sample. Peaks: (a) resveratrol; (b) quercetin; (c) volumetric standard, genistein.

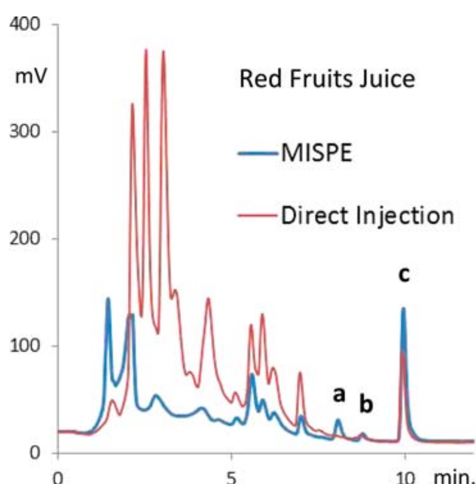

Figure 4. Chromatograms corresponding to analysis of untreated red fruits juice (red line) and red fruits juice MISPE extract (blue line). Peaks: (a) resveratrol; (b) quercetin; (c) volumetric standard, genistein.

wine and fruit juice are overlapped to the analysis of the correspondent amount of untreated sample. A much cleaner baseline was obtained, generating a lower LOD.

Finally, the presented method was applied to a few exemplary real samples, namely, red wine, "red fruits" juice, and lingonberry (*Vaccinium vitis-ideae*, Ericaceae; see Figure 5) juice, selected because of their high contents of (*E*)-resveratrol and quercetin.[35] The results of the real samples analysis (reported in Table 5) are

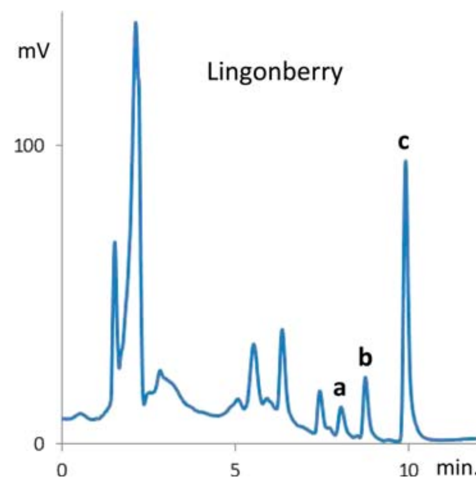

Figure 5. Chromatograms corresponding to analysis of lingonberry juice drink. Peaks: (a) resveratrol; (b) quercetin; (c) volumetric standard, genistein.

compatible with the concentration expected from the literature.[35,36]

Table 5. (*E*)-Resveratrol and Quercetin (More Relevant Natural Polyphenols Antioxidant) Content in Three Beverages Determined by MISPE HPLC-UV

	resveratrol		quercetin	
	mg L^{-1}	SD	mg L^{-1}	SD
wine	0.946	0.007	0.475	0.010
red fruits juice	0.034	0.001	0.868	0.016
lingonberry	0.161	0.010	1.871	0.087

The presence of a few peaks corresponding to strongly retained compounds in real samples warns about the possibility of using MISPE in the untargeted analysis of specific pharmacophores.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

4-VP, 4-vinylpyridine; AIBN, α,α'-azoisobutyronitrile; EGDMA, ethylene glycol dimethacrylate; ESI, electrospray ionization; HPLC, high-performance liquid chromatography; IF, imprinting factor; LOD, limit of detection; MDL, method detection limit; MIP, molecularly imprinted polymer; MISPE, molecularly imprinted solid phase extraction; MS, mass spectrometry; NIP, nonimprinted polymers; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; SD, standard deviation; SPE, solid phase extraction; UV, ultraviolet

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